# Epicardial, paracardial, and perivascular fat quantity, gene expressions, and serum cytokines in patients with coronary artery disease and diabetes

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Abstract: INTRODUCTION Obesity and diabetes mellitus (DM) are common disorders that increase cardiovascular risk and lead to coronary artery disease (CAD). OBJECTIVES The aim of our study was to assess the link between epicardial fat (EF) volume and paracardial fat (PF) volume, relative expressions of several genes in epicardial, paracardial, and perivascular fat and corresponding serum cytokines in patients with CAD in relation to DM. PATIENTS AND METHODS A total of 66 consecutive patients (33 with DM) with multivessel CAD were included. We obtained cardiac magnetic resonance, serum cytokines levels, and their relative mRNA expressions in EF, PF, and perivascular fat samples of the following: adrenomedullin (ADM), fibroblast growth factor 21 (FGF21), transforming growth factor (TGF ), phospholipid transfer protein (PLTP), receptor for advanced glycation endproducts (RAGE), thrombospondin 1 (THSB1), and uncoupling protein 1 (UCP1). RESULTS There were no differences in the anthropometric parameters or fat depots, except for higher epicardial fat volume in patients with DM (mean [SD], 105.6 [38.5] ml vs 84 [29.2] ml; P = 0.02). Patients with DM exhibited a significantly increased RAGE expression in EF (median [Q1-Q3], 0.17 [0.06-1.48] AU vs 0.08 [0.02-0.24] AU, P = 0.03). Diabetes was also associated with increased expression of ADM in EF and PF and decreased expression of FGF21 compared with patients without DM. CONCLUSIONS Patients with multivessel CAD and DM revealed increased volume and more dysfunctional profile of gene expressions in EF and significantly decreased expression of cardioprotective FGF21.

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ORIGINAL ARTICLE

# Epicardial, paracardial, and perivascular fat quantity, gene expressions, and serum cytokines in patients with coronary artery disease and diabetes

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**KEY WORDS**

adipose tissue dysfunction, coronary artery disease, diabetes, epicardial fat, perivascular fat

**EDITORIAL**

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**ABSTRACT**

**INTRODUCTION** Obesity and diabetes mellitus (DM) are common disorders that increase cardiovascular risk and lead to coronary artery disease (CAD).

**OBJECTIVES** The aim of our study was to assess the link between epicardial fat (EF) volume and paracardial fat (PF) volume, relative expressions of several genes in epicardial, paracardial, and perivascular fat and corresponding serum cytokines in patients with CAD in relation to DM.

**PATIENTS AND METHODS** A total of 66 consecutive patients (33 with DM) with multivessel CAD were included. We obtained cardiac magnetic resonance, serum cytokines levels, and their relative mRNA expressions in EF, PF, and perivascular fat samples of the following: adrenomedullin (*ADM*), fibroblast growth factor 21 (*FGF21*), transforming growth factor β (*TGF*β), phospholipid transfer protein (*PLTP*), receptor for advanced glycation endproducts (*RAGE*), thrombospondin 1 (*THSB1*), and uncoupling protein 1 (*UCP1*).

**RESULTS** There were no differences in the anthropometric parameters or fat depots, except for higher epicardial fat volume in patients with DM (mean [SD], 105.6 [38.5] ml vs 84 [29.2] ml; $P = 0.02$). Patients with DM exhibited a significantly increased *RAGE* expression in EF (median [Q1–Q3], 0.17 [0.06–1.48] AU vs 0.08 [0.02–0.24] AU, $P = 0.03$). Diabetes was also associated with increased expression of *ADM* in EF and PF and decreased expression of *FGF21* compared with patients without DM.

**CONCLUSIONS** Patients with multivessel CAD and DM revealed increased volume and more dysfunctional profile of gene expressions in EF and significantly decreased expression of cardioprotective *FGF21*.

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**INTRODUCTION** Obesity and diabetes (DM) are common disorders that increase cardiometabolic risk and can lead to cardiovascular diseases.[1,2] Precise evaluation of cardiovascular risk related to obesity and DM depends on the quantity, distribution, and location of body fat and the function of adipocytes in fat depots as well as in the entire body.[3,4] Epicardial fat (EF), paracardial fat (PF), and perivascular fat (PVF) are specific visceral fat depots whose role in cardiovascular diseases is well established.[5] Epicardial fat is embryologically different from other depots; it has no anatomical boundaries with the heart muscle or coronary arteries.[5] Recent studies have suggested its active paracrine and endocrine role in coronary artery disease (CAD).[6-8] The pathophysiological

## WHAT'S NEW?

We present a comprehensive study of epicardial, paracardial, and perivascular fat depot quantification, genes expressions, and corresponding serum cytokines in patients with and without diabetes (DM). Despite similar anthropometric parameters of obesity, patients with DM had increased epicardial fat volume with no differences in other fat depots. Diabetes is associated with decreased mRNA relative expression of FGF21, increased expression of ADM and RAGE in epicardial fat suggesting their unique role in atherosclerotic pathway in DM in contrast to TGFβ, PLTP, THSB1, or UCP1. Finally, serum cytokines levels do not follow the relative mRNA expressions of the corresponding cytokines in fat depots, which limits their potential role as markers of adipose tissue dysfunction.

mechanisms responsible for the balance between protective and proatherosclerotic effects of EF are also not yet understood. Therefore, we aimed to evaluate the link between EF volume (EFV) and PF volume, relative expressions of several genes in EF, PF, and PVF and corresponding serum cytokines in patients with multivessel CAD in relation to DM. We hypothesized that EF, PF, and PVF samples obtained from patients with DM would have a more proatherogenic gene expression profiles, which would be associated with serum cytokines and increased fat volumes.

## PATIENTS AND METHODS

**Study population** A total of 66 consecutive patients (aged 45–80 years) with multivessel CAD scheduled for elective coronary artery bypass grafting (CABG) in the Department of Cardiac Surgery in Medical University of Silesia in Katowice were included in the study after consideration of exclusion criteria. Given the aim of the study, the main exclusion criteria were as follows: acute coronary syndrome at the index hospitalization and in prior 6 months, prior thorax surgery, indications for any other type of cardiac surgery, significant heart valve dysfunction, congestive heart failure, left or right ventricle systolic dysfunction, significant diastolic dysfunction, infectious diseases in prior 2 months, chronic inflammatory diseases, neoplastic diseases (diagnosis and / or treatment in prior 5 years), significant renal or liver dysfunction, any anti-inflammatory or anti-infective medicines in prior month, secondary causes of obesity, specific interventions aimed at obesity in prior 12 months, a 10% unintentional weight loss in prior 3 months or risk of malnutrition (Mini Nutritional Assessment score <12 points),[9] a very poor image quality or contraindications to cardiac magnetic resonance (CMR), known genetic predisposition for cardiovascular diseases.

**Study protocol** The study protocol included: a detailed clinical assessment (Supplementary material, *Table S1*) with evaluation of anthropometric parameters and adiposity, CMR performed for the volumetry of fat depots, peripheral venous blood samples for serum levels of cytokines, and fat samples obtained during cardiac surgery.

Patients were recruited and completed the study at the Department of Cardiology at the Medical University of Silesia. The study protocol was approved by the local Medical University of Silesia Ethic Committee (no. KNW/0022/KB1/127/I/13/14) and all patients gave their written informed consent to the protocol. This work was supported by the research non-commercial grant from Medical University of Silesia (KNW--1-085/N/5/0, KNW-1-016/K/8/K) and Polish Cardiac Society (Servier 2016).

**Clinical characteristics** Hyperlipidemia, hypertension, DM, and metabolic syndrome were identified based on prior diagnosis or current treatment and defined according to the guidelines.[10] Overweight and obesity were classified according to body mass index ([BMI], calculated as follows: BMI = body mass [kg] / height [$m^2$]) as normal weight (18.5–24.9 kg/$m^2$), overweight (25.0–29.9 kg/$m^2$), and obesity (≥30.0 kg/$m^2$), divided into class 1 (30.0–34.9 kg/$m^2$), class 2 (35.0–39.9 kg/$m^2$), and class 3 (≥40.0 kg/$m^2$). Waist circumference (midpoint between the lowest rib and the iliac crest) was measured with a tape measure at the end of expiration. Additional measures were also obtained: neck circumference just below the laryngeal prominence, midpoint thigh circumference, and midpoint arm circumference, and the sum of 3 parameters was calculated. Skinfolds measured the thickness of skin and subcutaneous fat with the use of the equations of Jackson and Pollock.[11] The bioelectric impedance analysis (Bodystat 1500, Bodystat, Isle of Man, United Kingdom) was used to measure the patients' body composition with body fat percentage.

Ultrasound (3.5 MHz transducer, GE Vivid 9, GE Healthcare, Milwaukee, Wisconsin, United States) was used in all patients to obtain the index measure of visceral abdominal fat.[12] All participants completed a detailed dietary assessment with a country-specific, validated food frequency questionnaire.[13] Afterwards, it was converted into nutrients with a country-specific database used in the multicenter PURE (Prospective Urban Rural Epidemiology) study.[14]

**Cardiac magnetic resonance imaging of epicardial and paracardial fat** We quantified EFV and PFV on electrocardiogram-gated cine images acquired on 1.5T system (GE Optima MR450w, GE Healthcare) with a dedicated cardiac coil using a steady-state free precession sequence. The scan parameters used were time to echo / time of repetition, 1.9/4.3 ms, slice thickness of 8 mm (no interslice gap). EF and PF were defined as the particular adipose tissue in end--diastole (EF, tissue between myocardium and visceral layer of pericardium and PF, all the fat surrounding the heart outside the EF) along the walls of the right and left ventricles starting from the basal segments (level of atrioventricular valves) up to the apex area.

Adipose tissue areas were traced manually using short-axis images on the zoomed images proceeding through the slices from the atrioventricular valves to the left ventricular apex and were multiplied by the slice thickness (Supplementary material, *Figure S1*). Afterwards, EFV and PFV were calculated using the summation of the disc method.

**Blood collection** Blood samples were collected from all the patients prior to cardiac surgery. Afterwards, serum was obtained by centrifugation and the samples were stored in aliquots at –80°C. The serum concentrations of the following cytokines (enzyme-linked immunosorbent assay [ELISA] kits) were performed according to the manufacturer's protocols: proadrenomedullin (ADM, Cucabio Biotech Co, Houston, Texas, United States), fibroblast growth factor 21 (FGF21, BioVendor, Brno, CZ), transforming growth factor β (TGFβ, Diaclone SAS, Besancon, France), phospholipid transfer protein (PLTP, Cloud-Clone Corp, Houston, Texas, United States), thrombospondin 1 (THSB1, Cloud-Clone Corp, Houston, United States), uncoupling protein 1 (UCP1, Cusabio Biotech Co, Houston, Texas, United States).

**Adipose tissue collection** The adipose tissue samples were taken in the beginning of CABG procedure in tissues that had not been previously traumatized. All fat samples were taken in the same order and in the same locations in all patients: 1) subcutaneous fat at sternotomy; 2) PF within thorax; 3) PVF from internal mammary artery; and 4) EF adjacent to the proximal right coronary artery (Supplementary material, *Figure S2*). The biopsy specimens were immediately stored at –80°C until further analysis.

**RNA extraction and reverse transcriptase-polymerase chain reaction** RNA was extracted using the phenol-chloroform method with TRI reagent (MRC Inc., Cincinnati, Ohio, United States).[15] Adipose tissue samples (200 mg each) were put into 2 ml Eppendorf-type tubes directly into 1 ml of TRI reagent, which contained 5 µl of precipitation carrier reagent (MRC Inc.). Thereafter, samples were homogenized by means of a rotor / stator, hand-held homogenizer (IKA-Werke GmbH & Co KG, Staufen, Germany). All subsequent steps were performed according to the manufacturer's instruction. Finally, purified RNA was resolved in 100 µl of nuclease-free water. RNA concentration was determined spectrophotometrically by measuring the absorbance at 260 nm (BioPhotometer, Eppendorf, Hamburg, Germany). A 500 ng of total RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Warsaw, Poland) in a reaction volume of 20 µl. Finally, the reverse transcription reaction mixture was diluted at a ratio of 1:4 with nuclease-free water. Quantitative analysis of the above-mentioned genes was carried out by a 2-step reverse transcriptase real-time quantitative polymerase chain reaction assay (QPCR). Human glyceraldehyde 3-phosphate dehydrogenase gene (*GAPDH*) was used as a reference for quantitative analysis, and 2 µl of the reverse transcription reaction mixture (ie, an equivalent of 10 ng of total RNA) was used as a template for QPCR. Real-time QPCR was performed using SYBR Select Master Mix (Thermo Fisher Scientific) in a total volume of 20 µl that contained 200 nm of each (forward and reverse) gene-specific primers. All primers for QPCR were derived from PrimerBank database (https://pga.mgh.harvard.edu/primerbank/).[16] All primer pairs spanned the intron / exon boundary to exclude the possibility of false-positive results from genomic DNA. Reactions were performed using Roche LightCycler 480 Instrument II (Roche Diagnostics, Warsaw, Poland) with a thermal profile set as follows: 94°C/3 min then 35 cycles of 94°C/30 s, 58°C/30 s, 72°C/45 s. The specificity of each reaction was confirmed by a melting-curve analysis. The increase in fluorescence was measured in real time and threshold cycle (Ct) values were obtained. The target gene Ct number was normalized to the endogenous reference *GAPDH* and the formula $2^{(-\Delta\Delta Ct)}$ was used to calculate relative (to subcutaneous fat) gene expression.[17]

**Statistical analysis** The results presented in the manuscript or tables are expressed as means (SD) for normally distributed variables, medians (quartiles Q1–Q3) for nonnormal distribution, or number (percentage). The distribution was tested for the normality with the Kolmogorov–Smironov test. Baseline clinical parameters and the measures were compared between the subgroups using the *t* test for normally distributed continuous variables; in case of nonnormal distribution, the Mann–Whitney test was used. Associations between parameters were assessed using the Pearson or Spearmen rank correlation analysis, depending on the parametric or nonparametric distribution of variables. A *P* value of less than 0.05 was considered significant. Statistical analysis was performed using the Statistica software (version 10.0, Stat Soft, Warsaw, Poland)

## RESULTS

**Study group characteristics** Our study group included 66 patients (mean [SD] age, 65.6 [7.2] years; men, 76%) with preserved left ventricular systolic function and a multivessel CAD scheduled for CABG. Patients clinical characteristics, adiposity, ultrasound, and CMR indices of fat depots are shown in Supplementary material, *Table S1*. In brief, the study patients had several cardiovascular risk factors, including hypertension and dyslipidemia in all individuals and the following: overweight (51%), obesity (38%), DM (50%), nicotinism (48%).

**Diabetes and multivessel coronary artery disease** The patients were divided into subgroups based on whether they had DM or not (TABLE 1). Patients with and without DM revealed very similar clinical

characteristics, which allowed us to search for any differences in the quantity of fat depots, local mRNA genes expressions, and corresponding serum cytokines concentrations.

There were no differences in the prevalence of cardiovascular risk factors, diet, clinical parameters of obesity and fat depots, except for EFV, which was increased in patients with DM (mean [SD], 105.6 [38.5] vs 84 [29.2] ml; $P = 0.02$) (TABLE 1).

**Serum profile of atherogenic cytokines** Serum PLTP levels were significantly higher in patients with DM compared with patients without DM (mean [SD], 18.2 [12.5–31.4] vs 12.9 [8.5–22] μg/ml; $P$=0.03). However, serum concentrations of the following cytokines were similar in both subgroups: ADM, FGF21, TGFβ, THBS1, UCP1 (TABLE 2).

**Relative mRNA genes expressions in epicardial, paracardial, and perivascular fat depots** Patients with DM showed a significantly higher *RAGE* expression in EF (mean [SD], 0.17 [0.06–1.48] AU vs 0.08 [0.02–0.24] AU; $P = 0.03$). Diabetes was also associated with increased expression of *ADM* in EF (mean [SD], 1 [0.46–2.2] AU vs 0.51 [0.14–1] AU; $P = 0.03$) and PF (mean [SD], 1 [0.22–0.6] AU vs 0.35 [0.13–0.9] AU; $P = 0.03$). Moreover, the expression of *FGF21* in EF was significantly decreased in patients with DM compared with those without DM (mean [SD], 2.6 [0.4–5.1] AU vs 4.2 [1.1–22.7] AU; $P = 0.04$) and PF (1.43 [0.9–3.2] AU vs 14.2 [0.55–27.8] AU; $P = 0.04$). However, there were no differences in relative expressions of *TGFβ, PLTP, THSB1*, and *UCP1* between both subgroups (TABLE 3).

Given that patients with DM had significantly increased EF depot (EFV), all the relative expressions of genes obtained from the EF samples were indexed to EFV and compared (TABLE 4).

A comprehensive correlation analysis was performed in the study group and subgroups with and without DM. EFV was associated with BMI (DM [+], $r = 0.4$; $P = 0.03$ and DM [–], $r = 0.4$; $P = 0.02$) and waist circumference (DM [+], $r = 0.4$; $P = 0.02$ and DM [–], $r = 0.4$; $P = 0.04$). Paracardial fat volume was associated with BMI (DM [+], $r = 0.55$; $P = 0.02$ and DM [–], $r = 0.45$; $P = 0.01$) and waist circumference (DM [+], $r = 0.5$; $P = 0.002$ and DM [–], $r = 0.4$; $P = 0.02$).

Among serum levels of cytokines, TGFβ was associated with EFV in patients with ($r = 0,5$; $P = 0.004$) and without DM ($r = 0.3$; $P = 0.03$). Moreover, relative expression of *UCP1* in EF was associated with EFV in patients with DM ($r = –0.5$; $P = 0.03$) and without DM ($r = –0.4$; $P = 0.05$). The expression of *ADM* showed association with EFV / PFV ratio in patients with DM ($r = 0.6$; $P < 0.001$) and a tendency toward association in patients without DM ($r = 0.3$; $P = 0.04$). The CMR quantity parameters of fat depots did not reveal associations with serum cytokines or mRNA expressions in corresponding fat depots.

There were significant associations of relative expressions of *FGF21, RAGE*, or *ADM* in different fat depots (Supplementary material, *Table S2*).

All other associations not mentioned above, including gene expressions of all 3 fat depots and serum concentrations of the corresponding cytokines were not significant (data not shown).

**DISCUSSION** Our study evaluated the quantity and quality of EF, PF, and PVF depots in relation to DM in patients with severe CAD. First, despite similar anthropometric parameters of obesity, patients with DM had increased EFV with no differences in other fat depots. Second, DM is associated with decreased mRNA relative expression of *FGF21* (EF and PF), increased expression of *ADM* (EF and PF) and increased expression of *RAGE* in epicardial adipose tissue suggesting their unique role in atherosclerotic pathway in DM in contrast to *TGFβ, PLTP, THSB1*, or *UCP1*. Finally, serum levels of cytokines do not follow the relative mRNA expressions of the corresponding cytokines in fat depots, which limits their potential role as markers of adipose tissue dysfunction.

To the best of our knowledge, this is the first study providing a comprehensive CMR assessment and profiles of gene expressions in 3 fat depots with serum concentrations of corresponding cytokines in multivessel CAD, which suggests unique pathophysiological findings for DM.

**Fat depot quantity** In our study, patients with multivessel CAD and DM showed significantly increased EF compared with those without DM, which is in line with previous reports.[18-20] None of the other fat depots or anthropometric parameters of obesity revealed any differences between both subgroups. There are several studies focused on histological analysis of fat samples obtained mainly in larger visceral fat depots or individuals with severe obesity in relation to obesity or metabolic dysfunction.[21-23] Increased EFV results from various depot-specific histological findings, including a simple hypertrophy and hyperplasia in response to excess energy in obesity to inflammatory cells infiltration and different patterns of fibrosis in DM and dyslipidemia.[22] The increase in adiposity is associated with enhanced inflammation[23] and adipocyte size, and distribution is also linked with insulin resistance and DM, but evidence regarding causative associations is limited.[24] Patients in our study were in various BMI categories and some EF samples obtained from lean individuals were not enough for additional histological study. EFV showed only moderate associations with BMI and waist circumference and a moderate association with serum TGFβ, which was found in all patients. Moreover, EFV was inversely correlated with *UCP1* expression, especially in patients with DM. It suggests that the increased quantity of EF is more specific for metabolic disturbances, including DM, and not related to serum cytokines or mRNA genes expression involved in atherogenesis. UCP1 is a major marker

**TABLE 1** Clinical characteristics, anthropometric parameters, and adiposity in patients with and without diabetes

| Parameter | Multivessel coronary artery disease | | *P* value |
|---|---|---|---|
| | Diabetes (+) (n = 33) | Diabetes (–) (n = 33) | |
| Clinical characteristics | | | |
| Age, y, median (IQR) | 65 (61–73.5) | 65 (58–69) | 0.17 |
| Fasting plasma glucose, mg/dl, mean (SD) | 123.3 (9.3) | 89.2 (9.9) | <0.001 |
| Glycated hemoglobin, %, mean (SD) | 7.8 (0.8) | 5.95 (0.4) | <0.001 |
| Hypertension, n (%) | 33 (100) | 33 (100) | 1.0 |
| Dyslipidemia, n (%) | 33 (100) | 33 (100) | 1.0 |
| Total cholesterol, mg/dl, mean (SD) | 154 (38.3) | 155.6 (33.7) | 0.86 |
| LDL cholesterol, mg/dl, mean (SD) | 86.5 (32.4) | 90.1 (28.9) | 0.64 |
| HDL cholesterol, mg/dl, mean (SD) | 44.9 (12) | 42.7 (9.8) | 0.42 |
| Triglycerides, mg/dl, median (IQR) | 103 (84.7–141.7) | 108 (84–105) | 0.68 |
| Smoker or ex-smoker[a], n (%) | 18 (54) | 14 (42) | 0.33 |
| Risk factors[b], median (IQR) | 6 (6–7) | 4 (4–6) | <0.001 |
| Number of vessels with CAD[c], median (IQR) | 3 (2.75–3) | 3 (2–3) | 0.16 |
| LVEDV, ml, mean (SD) | 139 (35) | 154 (32.5) | 0.24 |
| LV mass, g, mean (SD) | 131 (37) | 136.2 (32) | 0.63 |
| LVEF, %, median (IQR) | 58.5 (53–60) | 55 (53.2–60) | 0.44 |
| Anthropometric parameters | | | |
| Overweight, n (%) | 15 (45) | 19 (57) | 0.33 |
| Obesity, n (%) | 14 (42) | 11 (33) | 0.45 |
| Weight, kg, median (IQR) | 85 (77–95) | 81.5 (73–92) | 0.28 |
| Body surface area, m², median (IQR) | 1.94 (1.8–2.1) | 1.87 (1.6–2.1) | 0.12 |
| Body fat[d], % | 33.7 (8.4) | 30.27 (6.5) | 0.11 |
| Body mass index, kg/m² | 29.9 (4.8) | 29.2 (4.3) | 0.47 |
| WC: W >80 or M >94, n (%) | 30 (90) | 29 (87) | 0.72 |
| WC, cm, median (IQR) | 102 (97.5–112) | 98 (92.5–111) | 0.33 |
| Neck + thigh + arm circumference, cm, median (IQR) | 116 (109–127) | 116 (105–125) | 0.62 |
| 3-sites skinfold thickness, mm, median (IQR) | 73 (63–79) | 65 (59–70.5) | 0.07 |
| Adiposity | | | |
| Abdominal visceral FAT, mm, median (IQR) | 49 (33.2–65) | 41 (28.7–56) | 0.52 |
| Epicardial fat volume, ml, mean (SD) | 105.6 (38.5) | 84 (29.2) | 0.02 |
| Pericardial fat volume, ml, median (IQR) | 121.2 (94.2–186.2) | 124.3 (84.5–161.7) | 0.91 |
| Epi-to-pericardial fat ratio, mean (IQR) | 0.83 (0.59–1.1) | 0.64 (0.53–0.84) | 0.07 |
| Thorax subcutaneous fat index, median (IQR) | 100 (77.6–120) | 95 (85.5–108) | 0.54 |
| Diet, median (IQR) | | | |
| Total energy intake, kcal | 1597 (1156–2318) | 1631 (1317–2043) | 0.91 |
| Carbohydrates, g | 205 (144–264) | 216 (163–251) | 0.54 |
| Lipids, g | 59.4 (43–100) | 54.5 (42–69) | 0.36 |
| Proteins, g | 67 (47–99) | 61 (53–74) | 0.62 |
| Cholesterol, mg | 239 (148–334) | 211 (153–293) | 0.32 |
| n-3 PUFA, g | 1.6 (1–2.1) | 1.4 (1–1.9) | 0.41 |
| n-6 PUFA, g | 8.4 (4.8–9.4) | 7.2 (5–8.9) | 0.52 |

**a** Current smoking or smoking in the past for at least 1 year

**b** Risk factors: male sex, age >55 years old, hypertension, hyperlipidemia, obesity, type 2 diabetes, chronic kidney disease, smoking

**c** Number of coronary arteries with ≥50% stenosis

**d** Body fat percentage assessed by bioelectrical impedance method

SI conversion factors: to convert fasting plasma glucose to mmol/l, divide by 18; cholesterol to mmol/l, multiply by 0.02586.

Abbreviations: CAD, coronary artery disease; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; M, men; PUFA, polyunsaturated fatty acid; W, women; WC, waist circumference

**TABLE 2** Serum cytokines in patients with and without diabetes

| Parameter | Multivessel coronary artery disease | | *P* value |
|---|---|---|---|
| | Diabetes (+) | Diabetes (–) | |
| Proadrenomedullin, pmol/l | 3.3 (1.7–4.8) | 2.4 (1.6–4.2) | 0.51 |
| Fibroblast growth factor 21, pg/ml | 209 (126–341) | 212 (73–328) | 0.42 |
| Transforming growth factor β, pg/ml | 6259 (6070–6826) | 6229 (6028–7174) | 0.73 |
| Phospholipid transfer protein, µg/ml | 18.2 (12.5–31.4) | 12.9 (8.5–22) | 0.03 |
| Thrombospondin 1, ng/ml | 1537 (1312–1570) | 1540 (1444–1567) | 0.92 |
| Uncoupling protein 1, pg/ml | 352.2 (184–585) | 299.2 (107–560) | 0.45 |

Data are presented as median (interquartile range).

**TABLE 3** Relative gene expressions of mRNA in fat depots of patients with and without diabetes

| Parameter | Multivessel coronary artery disease | | *P* value |
|---|---|---|---|
| | Diabetes (+) | Diabetes (–) | |
| Epicardial fat | | | |
| Advanced glycation end products rec. | 0.17 (0.06–1.48) | 0.08 (0.02–0.24) | 0.03 |
| Proadrenomedullin | 1.0 (0.46–2.2) | 0.51 (0.14–1) | 0.03 |
| Fibroblast growth factor 21 | 2.6 (0.4–5.1 | 4.2 (1.1–22.7) | 0.04 |
| Transforming growth factor β | 0.14 (0.05–0.5) | 0.1 (0.03–0.3) | 0.31 |
| Phospholipid transfer protein | 1.9 (1.1–3.1) | 1.4 (0.9–3) | 0.35 |
| Thrombospondin 1 | 0.57 (0.25–1.8) | 0.54 (0.2–1.1) | 0.61 |
| Uncoupling protein 1 | 1.5 (0.35–3.9) | 2.8 (0.9–5.5) | 0.32 |
| Pericardial fat | | | |
| Advanced glycation end products rec. | 0.15 (0.05–0.36) | 0.16 (0.03–1.25) | 0.61 |
| Proadrenomedullin | 1.0 (0.22–0.6) | 0.35 (0.13–0.9) | 0.03 |
| Fibroblast growth factor 21 | 1.43 (0.9–3.2) | 14.2 (0.55–27.8) | 0.04 |
| Transforming growth factor β | 0.13 (0.05–0.4) | 0.1 (0.04–0.22) | 0.32 |
| Phospholipid transfer protein | 1.63 (0.77–2.6) | 0.95 (0.4–2.1) | 0.22 |
| Thrombospondin 1 | 1.2 (0.55–1.6) | 0.75 (0.3–2.1) | 0.35 |
| Uncoupling protein 1 | 0.7 (0.2–3.2) | 1.31 (0.8–3.6) | 0.15 |
| Periarterial fat (IMA) | | | |
| Advanced glycation end products rec. | 0.04 (0.02–0.1) | 0.045 (0.02–0.08) | 0.71 |
| Proadrenomedullin | 1.0 (0.4–1.8) | 0.88 (0.3–1.1) | 0.14 |
| Fibroblast growth factor 21 | 0.8 (0.3–2.1) | 1.72 (0.3–9.7) | 0.21 |
| Transforming growth factor β | 0.23 (0.04–0.44) | 0.15 (0.08–0.5) | 0.72 |
| Phospholipid transfer protein | 1.3 (0.3–2.2) | 1 (0.4–2.5) | 0.91 |
| Thrombospondin 1 | 0.72 (0.28–2.25) | 0.35 (0.16–1.95) | 0.21 |
| Uncoupling protein 1 | 1.7 (0.9–3.7) | 4.76 (0.9–22.2) | 0.11 |

Data are presented as median (interquartile range). Gene mRNA expression in particular fat depot relative to its expression in subcutaneous fat (for details, see Patients and methods). All values are given in arbitrary units.

Abbreviations: IMA, internal nammary artery; rec., receptor

of brown adipose tissue (BAT), which is also involved in rapid thermogenesis and it was also found in EF among adults.[25] Our results showed no differences in *UCP1* expression in EF between patients with and without DM, which suggests that there is no difference in BAT-like phenotype in DM.[26] An inverse relation between EF expression of *UCP1* and EFV may be a result of a simple compensatory mechanism driven by a demand for thermogenesis.[25] However, evidence on *UCP1* expression in EF in relation to risk factors and EFV is ambiguous. Sacks et al[27] showed no significant association between *UCP1* expression and EFV or DM.

**Epicardial fat genes expressions** Epicardial fat is a unique depot with several local and systemic effects.[3] We aimed to assess the associations between various pro- and antiatherogenic cytokines that had the most limited evidence from previous studies.[5] Our study is the first to show that *FGF21* expression in visceral fat depots of patients with multivessel CAD and DM is significantly lower compared with individuals without

**TABLE 4** Relative gene expressions of mRNA in epicardial fat indexed to epicardial fat volume in patients with and without diabetes

| Parameter | Multivessel coronary artery disease | | *P* value |
|---|---|---|---|
| | Diabetes (+) | Diabetes (–) | |
| Advanced glycation end products rec. | 0.18 (0.37–0.88) | 0.08 (0.02–0.29) | 0.13 |
| Proadrenomedullin | 1.2 (0.5–2) | 0.7 (0.07–1.4) | 0.06 |
| Fibroblast growth factor 21 | 0.72 (0.08–4.1) | 2.1 (0.08–21.5) | 0.18 |
| Transforming growth factor β | 0.06 (0.01–0.2) | 0.04 (0.01–0.4) | 0.88 |
| Phospholipid transfer protein | 1.06 (0.2–2.1) | 1.07 (0.13–2.65) | 0.66 |
| Thrombospondin 1 | 0.35 (0.01–0.72) | 0.36 (0.06–1.5) | 0.65 |
| Uncoupling protein 1 | 0.27 (0.03–2.3) | 1.8 (0.1–5.6) | 0.12 |

Data are presented as median (interquartile range). All values are given in arbitrary units.

DM. Decreased expression of *FGF21* was found in EF and PF depots, which suggests that the lower expression of cardioprotective cytokine is a specific finding for DM, but not limited to epicardial (= pericoronary) fat as it is also found in other visceral fat depot. There were no differences in serum levels of FGF21 between patients with and without DM. Our finding suggests that future clinical trials aimed at FGF21 therapy should be focused on that subgroup of patients.

Fibroblast growth factors are a family of cytokines with multiple intracellular, paracrine, and endocrine effects on the cardiovascular system and metabolic pathways. FGF21 is a new and most promising cytokine in this family due to suggested cardioprotective role in cardiovascular diseases, including atherosclerosis.[28] Serum FGF21 levels were found to be increased in obesity, DM, metabolic syndrome, dyslipidemia, nonalcoholic fatty liver disease, and coronary or peripheral artery disease.[29] Researchers have speculated that increased serum levels of FGF21 may be an early biomarker of cardiometabolic dysfunction or it may reflect a compensatory cardioprotective response in pathological states.[29] Moreover, a growing body of evidence suggests that FGF21 is not only a cardiovascular marker but it plays some cardioprotective role. FGF21 was shown to exert lipid-lowering effects,[30] anti-atherosclerotic effects,[31,32] and cardioprotection in myocardial ischemia / reperfusion injury.[33] Moreover, the long-term treatment of FGF21 was shown to prevent diabetic cardiomyopathy.[34] Although the liver is a major source of serum FGF21, FGF21 pathways were also evidenced in adipose tissue[35] where it improves glucose uptake, lipolysis, oxidative capacity, and it affects thermogenic activity of BAT.[36] The evidence supporting cardiovascular and metabolic protective effects encouraged researchers to test the exogenous FGF21 in animal models and therapy in humans in clinical trials.[37,38]

*RAGE* is a multiligand receptor for advanced glycation end products and other peptides, which is involved in the inflammatory process, metabolic dysfunction, and atherosclerosis.[39] We found that patients with DM have significantly increased expression of *RAGE* in EF with no differences in PF and PVF depots compared with those without DM. Epicardial *RAGE* expression was not associated with the amount of epicardial depot as it was reported by Dozio et al[39] using echocardiography fat thickness. However, we used a reference method of CMR providing EFV, which is a much more precise parameter. *RAGE* may be involved in atherosclerosis in both DM and other conditions,[40,41] but our results suggest that it is associated with several proatherogenic pathways only in patients with multivessel CAD and DM.

*ADM* was shown to exert some protective effects on cardiac ischemia / reperfusion injury, vascular calcification or inflammation, it is also involved in obesity-related insulin resistance in experimental models.[42] Iacobellis et al[43] showed that *ADM* mRNA expression in EF was lower in patients with CAD than in those without CAD. Our study group included only patients with multivessel CAD and we found that *ADM* expression is upregulated in individuals with DM suggesting a compensatory mechanism. Alternatively, lower expression of protective *ADM* observed in patients with DM may be more pronounced in individuals without DM.

There were no differences between patients with and without DM in mRNA expression of *TGFβ, PLTP, THSB1* and *UCP1*. It suggests that those cytokines are not unique for coronary atherosclerosis in DM.

**Quality and function of epicardial fat** Although all study patients had a multivessel CAD, those with DM revealed a more pro-atherogenic profile of fat gene expressions suggesting that a pericoronary fat dysfunction may play a more important role in this subgroup. Our study protocol involved CMR as a reference method for EF quantification and other imaging methods may provide only an approximate measure of EF. Finally, we show that except for *UCP1*, none of the other gene expressions was related to EFV. However, relative gene expressions in EF were not different (except for tendency for *ADM*) between both subgroups when indexed to EFV. It implies that the differences in expressions of most genes found in patients with DM may be interrelated with the amount of EF.

Moreover, we also found that serum levels of cytokines involved in atherogenesis do not reflect their expressions in EF and serum concentrations cannot be used as simple markers of fat dysfunction. While pro and anti-atherogenic cytokines assessed in serum may reflect systemic or metabolic dysfunction, EF genes profile may reflect local fat dysfunction, which is a more important link between DM and multivessel CAD. Diet nutrients obtained with a very detailed PURE questionnaire were not associated with EF function.

**Limitations** Our results are based on a cross-sectional study and thus we cannot draw conclusions on causality and prospective changes in fat depots. However, our study design and methods used for fat specimen assessment are limited to cardiac surgery, which makes prospective observation nearly impossible. For the same reasons, we also do not compare our result with control healthy group as we believe that other clinical indications for cardiac surgery are always associated with some alterations in cytokines serum levels or gene expressions. Moreover, unlike previous studies, we compared and correlated serum concentrations and local gene expressions of corresponding cytokines and reference volumes of fat depots, including the PVF around internal mammary artery. However, due to difficulty and safety in obtaining EF specimens, the volume of specimens, especially in lean individuals without DM, hindered the assessment of genes at the protein level or histological analysis.

**Conclusions and clinical implications** Increasing prevalence of obesity, DM, and related adipose tissue dysfunction will be important triggers for cardiovascular diseases. Among patients with multivessel CAD, DM is associated with increased EFV and more dysfunctional profile of genes expression in EF. The amount of EF and mRNA genes expressions found in EF are not associated. Patients with DM revealed significantly decreased expression of cardioprotective FGF21 in EF and PF depots. Further clinical trials on exogenous FGF21 therapy should focus on patients with at least high cardiovascular risk and DM.

## SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

## ARTICLE INFORMATION

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**CONTRIBUTION STATEMENT** MH conceived the concept for the study, contributed to all parts of the study, drafted the manuscript; GM, ES, and BO performed all the serum an genetic lab tests; AK and MD obtained the fat samples; MB and GG analyzed the data; BRI performed diet analysis; RM improved CMR fat analysis; ZG improved the final manuscript. MH is the guarantor of this work and, as such, had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**CONFLICT OF INTEREST** None declared.



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